Universidade de Aveiro Departamento de Biologia
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FERNANDO JOSÉ DUARTE FERNANDES

Contribution to the Study of Cod *Gadus morhua*

Contribuição para o estudo do bacalhau *Gadus mohrua*

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Contribution to the Study of Cod *Gadus morhua*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica do Doutor Newton Carlos Marcial Gomes, Investigador Principal do Centro de Estudos do Ambiente e do Mar (CESAM) da Universidade de Aveiro.

Dedico este trabalho à minha esposa Helena, ao meu filho Pedro, à minha nora Ana e às minhas netas Inês e Constança, companheiras e companheiro nas longas horas de estudo e pesquisa.

o júri

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palavras-chave Bacalhau, pele, barbatana, comunidades bacterianas, DGGE

resumo

A rastreabilidade é uma das ferramentas mais usadas no controlo de origem de pescado e pode contribuir para a qualidade e segurança do produto. Contudo, até à data, os métodos usados na identificação da origem do pescado assim como das suas características químicas e biológicas envolve a perda de valor comercial do pescado amostrado. Saliente-se também que todos estes métodos são demasiado morosos e com custos dispendiosos para a indústria de transformação. Este trabalho foi realizado com o sentido de auscultar necessidades reais da indústria de transformação e secagem de bacalhau e numa perspetiva de desenvolvimento de um método eficiente e menos dispendioso de rastreabilidade de espécies de bacalhau salgado seco recorrendo a uma estratégia de PCR-DGGE.

keywords

Codfish, skin, fin, bacterial communities, DGGE

abstract

Traceability is one of the most used tools to control fish origin and can contribute to improve product quality and safety. However, until now the methods used to identify the fish origin and its chemical and biological characteristics involve the loss of commercial value of the fish sampled. It should be noticed also that all these methods are too time-consuming and have expensive costs for the manufacturing industry. This work was performed based on the real needs of the manufacturing and drying industry of codfish and in a perspective of developing an efficient and inexpensive method for traceability analysis of dry salted codfish species using a PCR-DGGE approach.

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1. INTRODUCTION

The food and drink industry is one of the most important and dynamic sectors in Europe and is represented by about 310 000 enterprises, providing jobs for more than 4 million people. Despite this high number, most are small enterprises that have no ability to compete with the global market. With an annual turnover of over 900 billion euros, this diverse sector is characterized by the large volume of its export and the development of numerous end products that are sold in domestic and international markets extremely competitive.

The falsification and the counterfeiting in the industry have not reached high proportions yet, however its level has been increasing. According to the Food Standards Agency, which supervises both food production and trade in the UK, reports of food frauds raise every year: in 2007, when the agency created its database, 49 complaints were lodged against 1,538 presented in 2014. According to the Committee on the Environment, Public Health and Food Safety of the European Parliament olive oil is the product more susceptible to be counterfeit. Though, in 2013 more than 1,000 tons of various foods were confiscated through an operation conducted by Interpol covering 33 countries. These foods involved fish and seafood, above all tuna, beverages, olive oil and vinegar, dairy products, among others. Thus, despite this illegal market is still in a growth phase, it is already taking alarming proportions. In fact, due to this growth several tests to products subject to counterfeiting are already being carried out. In the laboratory of West Yorkshire in the UK, several studies were carried out at this level and reported that in 900 food samples, 40% had changed their composition.

Food fraud is an issue that also encompasses the whole of Europe. For this reason the European Commission has taken a proactive role by creating a new unit responsible for food fraud in DG SANCO (Directorate-General for Health and Consumers). In 2013 the European Commission launched a five-point plan to combat existing fraud in the food industry and strength coordination between Member States and with other organizations such as Europol on issues targeted for falsification and counterfeiting in food. Apart from this plan new measures were imposed to create a greater control in the food distribution chains, such as unexpected check-ups of regulators along the food supply chain and the realization of mandatory testing. In addition it was also stipulated the allocation of strong penalties to those who practiced or contribute to the fraud of food.

Our food system is geared to mislead consumers. Thus, it is extremely important that consumers seek out and make sure that they are paying for what they really buy and not for food with less value and quality. According to the general principles of the law, food labeling is mandatory and must have name of the product, scientific name, origin, consumption date as well as allergenic included and ingredients used in conservation. The correct identification of fish species is an important issue to consider regarding their correct labeling, since labeling information is important to assist consumers to select foods correctly.

The labeling regulations for products derived from fisheries are increasingly more demanding, in order to ensure the traceability all along the chain and consequently avoid possible fraud. Thus, it is important to implement traceability from the start and create the possibility to verify it during the entire process until consumption time.

Recently many mislabeling scandals have occurred regarding the substitution of high commercial value fish species by lower value fishes, which is not so uncommon. This problem seems to be amplified when it touches to the profits involved in specific markets as codfish market. It´s not unusual to have *Theragra chalcogramma* and *Gadus macrocephalus* labelled as *Gadus mohrua*. This problem takes huge proportions along the entire chain amplified by all the intervenients, allowing fraud to be committed by distributors and restaurants. Authorities seem to have the problem of looking in the wrong direction, since they only act in consequence of complains or when some serious health problem occurs. However, in fact, identification is difficult in the case of organisms with morphological traits eliminated during processing, or sectioned portions. For the case stated above it is necessary the development of methodologies that allow the identification of the species along the entire fish processing.

Consumer's trends involve towards ready-to-use products. Lack of time is one of the reasons identified for the preparation of food and food products. But when it touches to salted codfish the cultural reasons overlap to this fact. The main question is if the fish is correctly identified as to its origin and source.

Traceability and identification of salted codfish are big concerns for Portuguese consumers and for the Portuguese transformation industry also. Portuguese consumers mainly purchase dry salted codfish and bits, but there is also market for traditional green salted by-products, such as codfish faces, cheeks, tongues, spines and "samos" (swimming bladder). Due to some differences related with morphology and coloration, consumers can at first glance, and if the fish is complete, distinguish if it is from the Atlantic or from the Pacific or if it is other gadoid species. Others consumers, more informed, can even distinguish the origin of Atlantic codfish, and choose between Canadian, Norwegian and Icelandic codfish. For the Portuguese industry of salted codfish, and in all stages (processing, distribution or selling), codfish can be identified using traceability, as a safety way to prevent fraud and further expensive lawsuits.

There is a continuous demanding when exporting fish for foreign markets or even for the main Portuguese supermarket chain's, to certificate the origin of codfish on delivery act. However, when doubts and suspicions are raised, the origin of the codfish needs to be proved, involving usually an extreme expensive process. In turn, this process implicate the loss of an entire piece of fish, the need of keeping counterproofs and additional samples used on further analyses on certified laboratories.

Salt has been used since immemorial time to preserve and improve shelf life of fish and food. Salted codfish has traditionally been one of the highly appreciated species in Portugal; dry salting process can be natural or artificial (Fig. 1). Due to its excellent storage, stability and sensorial properties it plays an important role in the Portuguese diet in the preparation of many traditional dishes. Codfish *G. mohrua* is the most consumed and traded specie of Gadidae family in Portugal and mainly comes from North Atlantic countries, such as Norway and Iceland.

Consumption in dry salted, desalted frozen and salted by-products as bits, cod faces, tongues have a high expression in Portuguese economy. Renaming and mislabeling of salted codfish products are two big concerns for Portuguese transformation industry (Jacquet and Pauly 2008). Prices and production costs have tendency to be higher and allied to speculation determine markets. Some not good practices and the lack of surveillance in markets are determining a new look on traceability by industries. In fact, traceability is a well known tool to ensure that products can be followed up and consumed safely. Another concern is the costs for proofing and spoilage. However, up to now, laboratory tests are expensive and longstanding. Concerning spoilage there is a need of sectioning the fish, usually in the middle center, which leads to the lost of its commercial value. Not relying on the necessity of having counterproofs from the same lot or lots that have potential problems.

Difficulties of an extremely competitive market coupled with significant annual costs with spoilage, proofing, counterproofs storage and laboratory tests are the reasons for this work.

Figure 1 - Dry salting process; a) natural or b) artificial. Images obtained by courtesy of Mr José Cachide.

The overall objective of this research is to contribute to the improvement of fish origin control in codfish industry. In line with this goal, a molecular approach for codfish traceability was evaluated in this study; here we tested codfish's skin and fins as a way to distinguish the origin of *G. mohrua*; as well after dried gadoid skin to detect its potential source (different fish species).

2. MATERIAL AND METHODS

The present study was carried out with the collaboration of two Portuguese companies headquartered in Gafanha da Nazaré, Portugal. They import Norwegian and Icelandic green salted codfish, and then process and dry for domestic and foreign markets.

2.1. SAMPLING AND DNA EXTRACTION

2.1.1. GREEN SALTED CODFISH

Small sections of skin and fins from salted codfish were used and with that avoiding the loss of commercial value of fish. The skin was collected between the cloacae and the caudal fin, into sections of about 20mm long by 3mm wide, while complete pelvic fins were collected.

Samples of green salted *G. morhua* were directly collected from two importers. Randomly twenty samples were collected from Norway and Iceland fishes. In total there were collected five samples of skin and five of fin from each origin (Norway and Iceland). Were collected from two fish sections, as is shown in Fig. 2; one sample of pelvic fin (Fig. 2a) and one of fish skin (Fig. 2b), removed aseptically and kept separated into Falcon tubes.

Figure 2 – Sampling areas of the green-salted *Gadus morhua*; a) pelvic fin b) skin.

Samples were obtained from sealed pallets and before drying process (kept chilled chambers between 0 and 4ºC). Green salted codfish usually have about 51-58% moisture content and if properly stored a shelf life of 12 months, according to national specification (Ministério da Agricultura, Decreto Lei 25/2005). Falcon tubes were sealed in plastic bags, kept in cooled freezer between factory and laboratory and then stored at −20ºC until treatment.

Each sample of pelvic fin was treated by removing completely fin rays. From both fin and skin samples was used 1-1.2 g of weight. The samples were then disposed individually in new Falcon tubes with four glass beads of 2.85 – 3.45 mm (ROTH, Karlsruhe, DE). Samples were shaken (BIOSAN, Rocker Shaker MR12) after the addition of 18 mL of 0,85% saline solution. Then, after removing and discarding both skin/fin biomass and beads, the Falcon tubes were centrifuged for 20 min at 4000 rpm and 4ºC, and the supernatant was discarded. Finally, the pellet was resuspended using 1mL of 0,85% saline solution and transferred to 2 mL FastPrep tubes with 500 mg of glass beads. Afterward, E.Z.N.A.® Soil DNA Kit (Omega bio-tek, Norcross, GA USA) was used following the manufacturer's instructions. A final volume of 50 µl of DNA was obtained and stored at −20 °C until use.

2.1.2. DRY SALTED CODFISH AND POLLOCK

Samples of dry salted *T. chalcogramma* (TC), *G. morhua* (GM) and *G. macrocephalus* (GMC) were directly supplied by the Quality Department of a commercial enterprise which processes and trades dry salted gadoid fish species in Portugal. Each fish species belongs to a different fish lot. However, since the fish boats are usually of small and medium tonnage (up to 22 meters), fishing time greater than 24 h and consequently differences in fish post-harvest age (iced storage time) are unlikely or reduced. Sampled fish were obtained from refrigerated chambers (between 2 and 6 °C) after the dry salted process and were ready to be delivered for retailsale. Processed fish usually have about 45% moisture content and a shelf life, if properly stored, of 12 months, according to national specification (Ministério da Agricultura, Decreto Lei 25/2005). Collected samples were sealed in plastic bags, in the factory and transported immediately to the laboratory where they were kept at -20 ºC until processing. Four fishes were sampled and analyzed for each gadoid species studied (4 replicates). Each fish sample consisted of three randomly collected pieces of fish skin, removed aseptically from the caudal fin region and combined into a single tube (2.0 ml microcentrifuge tube) before total community DNA extraction.

Nucleic acids (total community - DNA) were extracted from approximately 1-1.5g of fish skin from each species. All samples were homogenized using FastPrep® (Qbiogene Inc., California, USA) bead-beating system in combination with a mixture of beads and extraction buffer SLX Mlus [E.Z.N.A.™ Soil DNA Kit (Omega Bio-Tek Inc., Norcross, Georgia, USA)]. The DNA extraction was done according to manufacturer´s instructions, as was done for green salted codfish.

2.2. PCR-AMPLIFICATION OF 16S RRNA GENE FRAGMENTS

In this study DGGE fingerprinting was used to determine compositional variation among bacterial communities in different samples. The V6-V8 regions of bacterial 16S rRNA gene fragments suitable for bacterial DGGE fingerprints of total microbial community DNA samples were obtained using a nested approach following Gomes et al. (2008).

The first PCR was performed using primers 27F and 1494R (Weisburg et al. 1991). For this PCR, a mixture for 25 µl was prepared containing 1 µl BSA (2mg/mL), 12.5 µl PCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA USA), 0.25 µl primers 27F and 1494R and 1 µl DNA. Denaturation for 5 min at 94°C was carried out, after which 20 thermal cycles of 45 s at 94°C, 45 s at 56°C and 1 min 30 s at 72°C were performed. A final extension step of 10 min at 72°C finished the reaction.

One µl of the first PCR product was used as template for the second PCR, using primers 968GC and 1378R (Nübel et al. 1996). The GC clamp sequence was published elsewhere (Heuer et al. 1997). Reaction mixtures (25 µl) included 2 µl acetamide, 12.5 µl PCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA USA), 0.5 µl primers 968GC and 1378R. Denaturation for 4 min at 94°C was carried out, after which 25 thermal cycles of 1 min at 95°C, 1 min at 53°C and 1 min 30 s at 72°C were performed. A final extension step of 10 min at 72°C finished the reaction.

Both PCRs were conducted in a TProfessional TRIO Thermocycler (Biometra). Positive and negative controls were run for each PCR.

2.3. DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

DGGE gels of the amplified 16S rRNA gene sequences were performed using the DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). Samples were loaded onto 6-10% (w/v) polyacrylamide gel in 1 x Tris-acetate–EDTA buffer (TAE) with a denaturant gradient 40-58%, at 60 ºC and a constant voltage of 160 V for 16 h. The gel was silver-stained according to Byun et al. (2009), except for the stop solution that here was replaced by a Na_2CO_3 solution. The DGGE gel was scanned using the Epson perfection V700 Photo Scanner.

2.4. STATISTICAL ANALYSIS

DGGE gels were analysed with the software package BioNumerics v6.6 (Applied Maths, Belgium). Band standardization was carried out automatically by the program, but was always confirmed visually with changes made when necessary. Subsequently, the program constructed a matrix that incorporated the position and intensity of each band. Briefly, both band position and intensity were processed in Excel (Microsoft); and the band intensity was converted to relative intensity by dividing its intensity by the sum of all band intensities in a lane (sample).

A Bray-Curtis similarity index was calculated based on relative intensity of each band. Analysis of similarities (ANOSIM) and non-metric multidimensional scaling (MDS) were used to analyse DGGE profiles with PRIMER 5 (Primer-E Ltd, Plymouth UK). The ANOSIM was used to test if there is complete (R=1) or no (R=0) separation between bacterial communities from different samples (Clarke 1993). Significance was tested with a permutation test using 999 permutations (Clarke and Gorley 2001). Differences in bacterial community structure of both skin and fin samples were assessed graphically using MDS (Yannarell et al. 2005). Bray-Curtis similarity indexes were calculated based on the relative intensity of each band in a lane (sample) as a measure of similarity between the community fingerprints. Samples were grouped by applying the un-weighted pair group method with average linkages (UPGMA) to the matrix of similarities.

3. RESULTS

Evaluating of the number and position of bands in DGGE profiles allows determining whether skin or fins represent a better tool to distinguish between codfishes' origin. The DGGE gel shows the differences between bacterial communities from skin and fin samples from both Norway and Iceland (Fig. 3). It is clear that there are great similarities between both skin and fin Norwegian samples and fin Icelandic samples. On the other hand, Fig. 3 also shows that Icelandic skin samples are absolutely different from the rest of the samples, indicating skin as a better tool to distinguish codfishes' origin than fins.

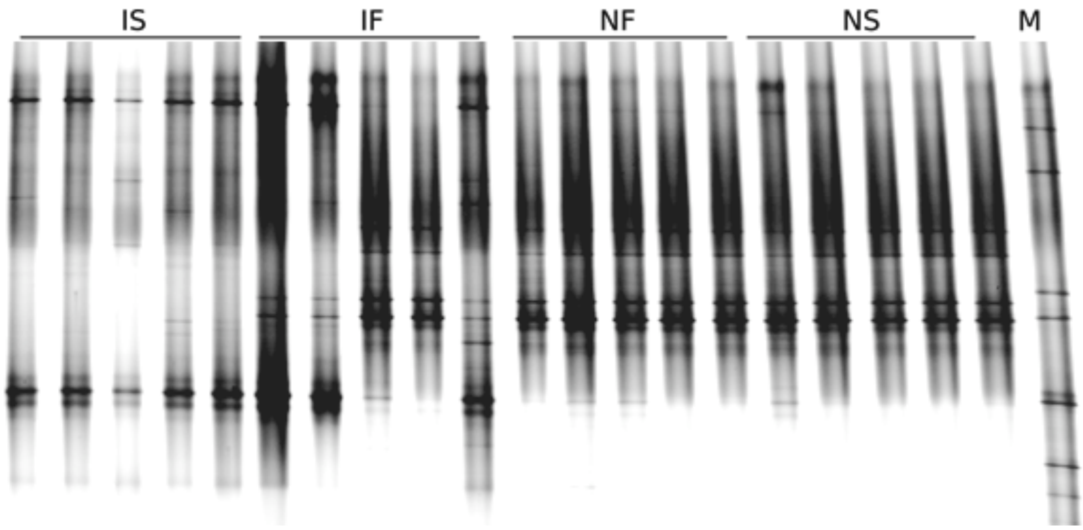

Figure 3 – Comparison of denaturing gradient gel electrophoresis (DGGE) patterns of 16S ribosomal RNA gene fragments of bacteria amplified from skin (S) and fin (F) from two origins (Norway - N and Iceland - I); M – DGGE marker.

Table 1 shows that, in general, there are significant differences between all the groups of samples (global R=0.601) and that there are significant differences among Icelandic skin samples and the other groups of the samples.

Table 1 – ANOSIM statistic analysis of Bray-Curtis distance (R) of denaturing gradient gel electrophoresis (DGGE) profiles of bacterial communities from Icelandic skin (IS) and fin (IF) samples and Norwegian skin (NS) and fin (NF) samples.

	Global R	Significance level %
	0.601	0.1
Group of samples	R Statistic	Significance level %
IS, IF	0.628	0.8
IS, NF	1	0.8
IS, NS	1	0.8
IF, NF	0.336	3.2
IF, NS	0.312	3.2
NF, NS	0.184	8.7

Non-metric multidimensional scaling (MDS) analyses corroborated ANOSIM results, indicating that samples from Icelandic skin are clearly separated from the other groups of samples (Fig. 4). It is also visible that Icelandic skin samples form a completely isolated cluster. Though, Icelandic skin samples are more separated from both Norwegian skin and fin samples than from Icelandic fin samples. Both Norwegian skin and fin samples also form clusters but that are barely separated from each other. MDS also agrees with ANOSIM showing than Icelandic fin samples are more separated from Icelandic skin samples than from both Norwegian skin and fin samples.

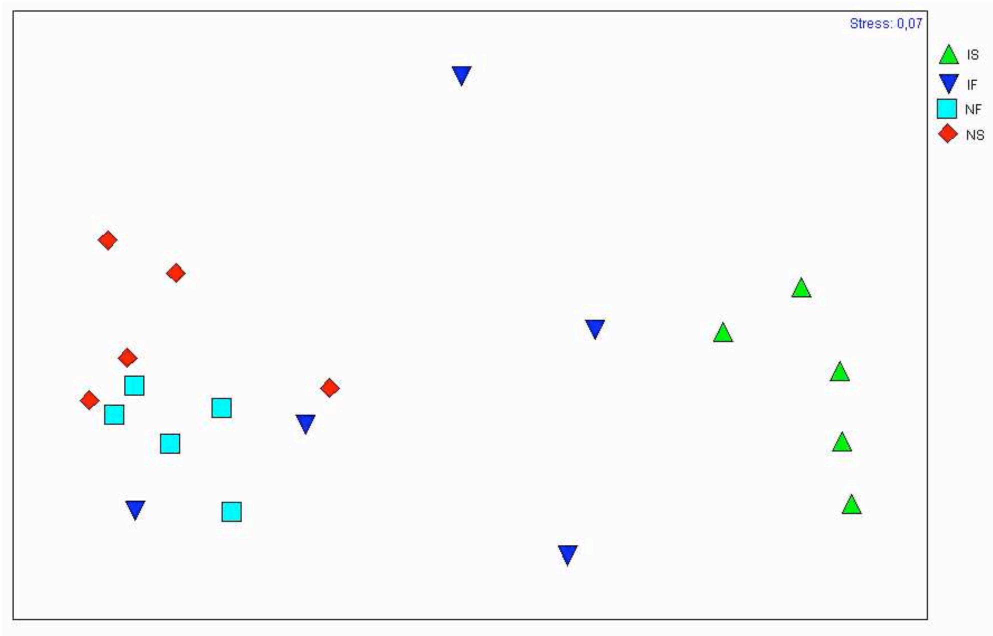

Figure 4 – MDS diagram of denaturing gradient gel electrophoresis (DGGE) profiles of bacteria amplified from skin (S) and fin (F) from two origins (Norway - N and Iceland - I).

One of the objectives of this study is to evaluate the bacterial communities of skin or fins as a marker to distinguish among the codfishes' origin. Through ANOSIM statistics and MDS analyses, it is clear that skin represents the best way to differentiate the origin of the codfishes (R=1). On the other hand, R-value between both Icelandic and Norwegian fins is 0.336, indicating that these groups barely separated.

In the case of dry salted gadoid species, the PCR-DGGE revealed significant differences in the structure and composition of skin bacterial assemblages. The DGGE gel shows differences between the three gadoid species, revealing the presence of different dominant bacterial populations (bands) and the presence of different less intense bands common to all treatments (Fig. 5). Additionally, there are bands common to *G. morhua* and *G. macrocephalus* but not to *T. chalcogramma* (Fig. 5).

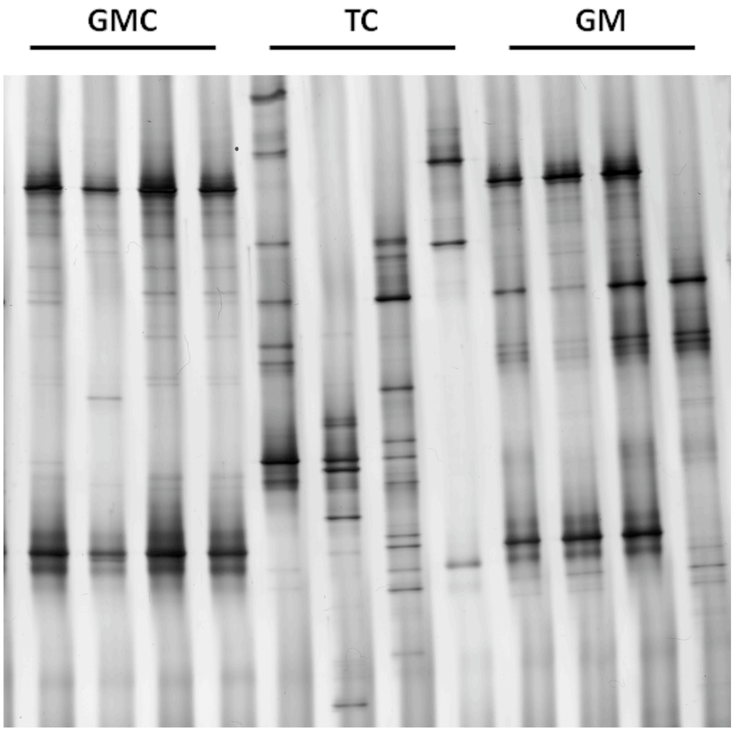

Figure 5 – Denaturing gradient gel electrophoresis (DGGE) fingerprint of 16S rRNA gene fragments amplified from four replicates of three different gadoid species: *Gadus macrocephalus* (GMC); *Theragra chalcogramma* (TC); *Gadus morhua* (GM) DNA templates are shown (Pegoraro et al. in press).

T. chalcogramma exhibited the highest variability of bacterial composition among all samples, which is corroborated by the similarity values shown in cluster analyses (Fig. 6). The cluster also shows the formation of two main clusters; where *G. macrocephalus* and *G. morhua* clusters are closer to each other (with exception of one sample) and more distant from *T. chalcogramma* cluster. In line with these results, ANOSIM results show the highest R values for *T. chalcogramma* vs. *G. macrocephalus* (R = 0.62) (separated) and *T. chalcogramma* vs. *G. morhua* (R = 0.48) (moderate differences); *G. macrocephalus* vs. *G. morhua* showed the lowest R values (R = 0.38) (barely separated).

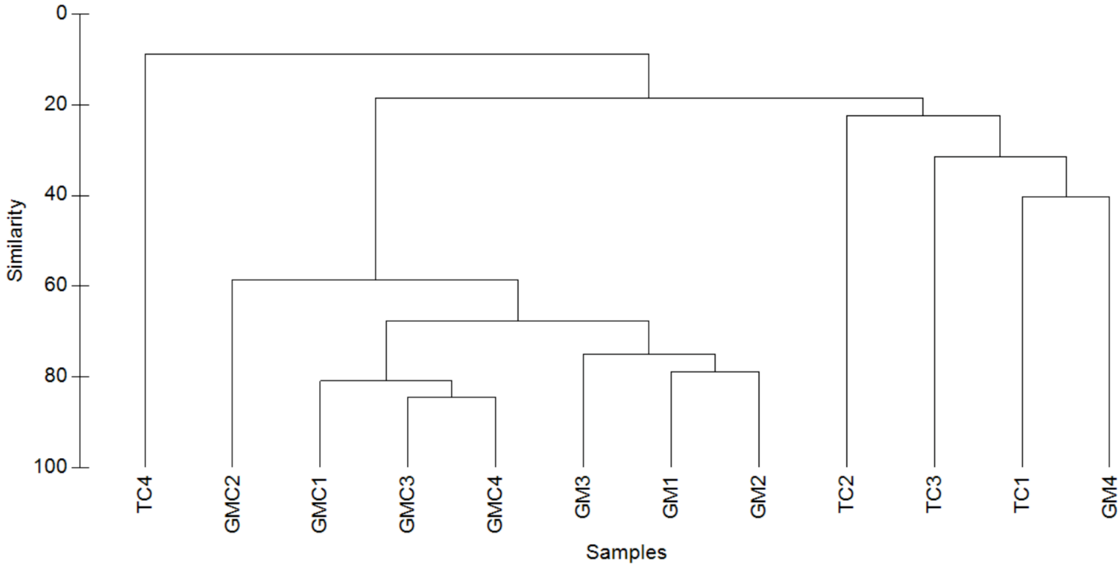

Figure 6 - UPGMA cluster analysis based on Bray-Curtis similarity index obtained from skin bacterial denaturing gradient gel electrophoresis (DGGE) profiles of *Gadus morhua* (GM), *Gadus macrocephalus* (GMC) and *Theragra chalcogramma* (TC).

Based on these results, after dry salting of gadoid species it is possible to distinguish *T. chalcogramma* from *G. macrocephalus* than from *G. morhua* and it is very difficult to differentiate the last two from each other, based only on PCR-DGGE approach.

4. Discussion

Traceability in food chain is being increasingly implemented worldwide. According to Ràbade and Alfaro (2006), traceability allows the coordination between all the chain intervenients and its main goal is to provide a full historic log (e.g., location) of the food. Recently, advances were made in this direction by the design of ICT (Information and Communication Technology) instruments, in order to facilitating data acquisition and reducing associated costs (Sahin et al. 2002, Regattieri et al. 2007, Gandino et al. 2009). The information collected by these systems is even more important when problems occur and the food has to be recalled (Bechini et al. 2008). Besides the negative impact that this may represent to a company's credibility it also may lead to undesired lawsuits. Bechini et al. (2008) proposed an effective traceability system to manage the relevant aspects related to inter-organizational information systems for complex supply chains and the important technical aspects specific to traceability.

On the other hand, falsification and counterfeiting in the food industry is increasing, targeting diverse food products. Consequently, consumers may be paying for less value and quality food. Recently, many cases of mislabeling were reported regarding the replacement of high commercial value fish by lower value fishes. In the case of codfish market there have been some cases where *G. morhua* has been replaced by *T. chalcogramma* or *G. macrocephalus*.

Production of dry salted codfish involves a very complex process, in which it is possible to have falsification and counterfeiting in several process points. Since the process has various intervenients, consequently it also has critical traceability points, where product or process information is lost or changed (Steinsträter and Jensen 2001, Bollen et al. 2007). Difficulty of fish identification increases when organisms with morphological traits are eliminated during processing, or are in sectioned portions. Not every consumer can distinguish the codfish origin, which leads to the necessity of the development of new methodologies of the identification of species along the entire fish processing. Industry of salted codfish is aware of this problem and is looking forward for less expensive and time-consuming process of codfishes' identification. In order to solve this, genetic techniques have been developed to allow the accurate identification of the codfish and avoid fraud. PCR-RFLP is one of the most used techniques in gadoid identification (Herrero et al. 2010 and references therein). This technique was also used to distinguish European anchovy from gilt sardine (Sebastio et al. 2001), hake baby foods, canned tuna (Ram et al. 1996, Quinteiro et al. 1998, Quinteiro et al. 1999, Quinteiro et al.

2001) and to identify two species of Atlantic horse mackerel (Takashima et al. 2006). Besides PCR-RFLP, RT-PCR has also been used to distinguish gadoid fishes (Herrero et al. 2010). Additionally, other molecular techniques have been used as traceability methods; AFLP markers that were used to identification and test authenticity of fish and seafood samples (Maldini et al. 2006), multiplex-PCR assay in the confirmation of canned Atlantic mackerel (Infante et al. 2006) and of fish fillets from grouper (Trotta et al. 2005). Sequencing methods were used in the identification of surimi-based products (Pepe et al. 2007) and molecular markers in processed anchovy products (Jérôme et al. 2008). Traceability methods have also been used in other food products beside fish and seafood products; such as DNA markers in olive oil (Pafundo et al. 2007) and in meat traceability (Arana et al. 2002).

The actual methods of codfish sampling involve the cut of a great amount of muscle mass, leading to the loss of its commercial value. In order to avoid the loss of an entire piece of codfish, in this study, we tested the possible use of a PCR-DGGE approach to distinguish different codfishes' origins. For this, bacterial communities of skin and pelvic fins of *G. morhua* from Iceland and Norway were compared. The analysis of DGGE profiles of bacterial communities associated to codfishes' skin and fins and the aftermost statistical analysis were done to evaluate if it is easier and more reliable to use skin or fins to differentiate codfishes' origin. The analysis of similarities (ANOSIM) of DGGE profiles was used to assess the differences between groups, i.e. Icelandic and Norwegian skin and fins; ANOSIM R value varies between 0 and 1, where higher values correspond to higher differences (Clarke 1993). The results obtained reveal significant differences (global R=0.601) between all the groups of samples. Low significant differences were found between both Norwegian skin and fins samples and Icelandic fins (R=0.312 and R=0.336, respectively), while Icelandic skin samples are completely separated from Norwegian skin and fins samples (both with R=1). These results suggest that besides being possible to use PCR-DGGE to distinguish the origin of green salted codfishes, this can be done reliably using green salted codfish skin, avoiding the loss of the codfish's commercial value.

Since dry salted products are rarely sterile, their microbial composition in the end of the process depends on the raw materials used, food processing parameters and subsequent storage conditions (Gram and Huss 1996, Rodrigues et al. 2003). Consequently, it is probable that codfish and pollock skin act as a source of microbial contamination during the entire industrial process. Handling is, therefore, a possible source of microbial contamination. In fact, based on previous studies, there are evidences that skin and mucus contribute for the growth of complex microbial communities and microbial cell abundance, respectively (Feng et al. 2010, Landeira-Dabarca et al. 2013). Feng et al. (2010) accessed the microbiota associated to skin, gills and intestines of yellow grouper fed with two different diets, proving that there were differences between intestinal-adherent bacteria samples and higher similarities among bacterial communities (DGGE based profiles) in skin and gills. The authors also suggest that the water from the rearing systems can be a source of microbes and influence the structural composition of microbial communities associated to the fishes. Landeira-Dabarca et al. 2013 also showed that both microbial density and community composition of epidermal mucous vary depending on the fishes' diet. They proved that food deprivation lead to a fast decrease in microbiota diversity and abundance associated to fishes' mucous and probably this is related with the reduction of mucous production.

In this study, we suggest that the differences in bacterial community profiles among gadoid species are due to the microbes naturally associated to their skin. Therefore, the differences between DGGE fingerprints from distinct dry salted gadoid fishes and the tendency to form distinctive clusters suggest that PCR-DGGE has potential to be used as a traceability method to distinguish dry salted gadoids from different origins, producers or species. However, since the differences among the three dry salted gadoid fishes were not so conclusive, further studies should be done concerning the use of other technologies combined with PCR-DGGE in order to investigate the use of this molecular technique as a traceability tool.

5. CONCLUSIONS

Due to the great consume and production of dry salted codfish and its by-products (codfish faces, cheeks, tongues, spines and “samos”), traceability and identification are of extremely importance for both Portuguese consumers and transformation industry. Therefore, this is essential to be able to distinguish between both fishes’ origin and species. Though, since the methods used hitherto are too expensive and time-consuming there is a need to develop an inexpensive and fast traceability method concerning dry salted codfish.

The results obtained here suggest that besides being possible to use a PCR-DGGE approach to distinguish different codfishes’ origins, this can be done reliably using codfish skin, avoiding the loss of an entire fish. Additionally, using this approach there is also a tendency to cluster gadoid species separately from each other. These findings indicate that such information can be used for development of molecular protocols for traceability of dry salted fish food products. However, further studies with larger number of samples from different origins are needed to confirm this trend. In addition more studies are needed to clarify the dynamics of fish skin bacterial flora after capture and during the dry salting process and its importance for fish traceability. Futures studies combining PCR-DGGE and other techniques may be done in order to develop DNA markers, allowing the identification of different gadoid fishes.

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